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PERMISSIVE ROLE FOR MGLU1 METABOTROPIC GLUTAMATE RECEPTORS IN EXCITOTOXIC RETINAL DEGENERATION

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Abstract—Neuroprotection is an unmet need in eye disorders characterized by retinal ganglion cell (RGC) death, such as prematurity-induced retinal degeneration, glaucoma, and age-related macular degeneration. In all these disorders excitotoxicity is a prominent component of neuronal damage, but clinical data discourage the development of NMDA receptor antagonists as neuroprotectants. Here, we show that activation of mGlu1 metabotropic glutamate receptors largely contributes to excitotoxic degeneration of RGCs. Mice at postnatal day 9 were challenged with a toxic dose of monosodium glutamate (MSG, 3 g/kg), which caused the death of >70% of Brn-3a^{+} RGCs. Systemic administration of the mGlu1 receptor negative allosteric modulator (NAM), JNJ16259685 (2.5 mg/kg, s.c.), was largely protective against MSG-induced RGC death. This treatment did not cause changes in motor behavior in the pups. We also injected MSG to *crv4* mice, which lack mGlu1 receptors because of a recessive mutation of the gene encoding the mGlu1 receptor. MSG did not cause retinal degeneration in *crv4* mice, whereas it retained its toxic activity in their wild-type littermates. These findings demonstrate that mGlu1 receptors play a key role in excitotoxic degeneration of RGCs, and encourage the study of mGlu1 receptor NAMs in models of retinal neurodegeneration. © 2017 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: retinal ganglion cells, mGlu1 receptors, monosodium glutamate, JNJ16258695, *crv4* mice.

INTRODUCTION

Retinal ganglion cells (RGCs) collect the visual information from photoreceptors *via* bipolar and amacrine cells, and transfer the information to the brain through the optic nerve. Excitotoxic degeneration of RGCs play a role in the pathophysiology of retinal disorders, such as glaucoma (Lipton, 2003; Seki and Lipton, 2008), age-related macular degeneration, and prematurity-induced retinal degeneration (Hinton et al., 1986; Tenhula et al., 1992; Lipton, 2003; Casson, 2006; Kaur et al., 2008; Seki and Lipton, 2008; Hernández and Simó, 2012). It is generally believed that glutamate-induced RGC damage is mediated by the activation of N-methyl-D-aspartate (NMDA) receptors and the resulting increase in intracellular Ca^{2+} (Ferreira et al., 1996; Niwa et al., 2016). However, the value of NMDA receptors as candidate drug targets for retinal neuroprotection was questioned by the disappointing clinical data with the NMDA channel blocker, memantine, in the treatment of glaucoma (Chidlow et al., 2007; Osborne, 2009). The lack of efficacy of memantine might reflect the opposite role of synaptic and extrasynaptic NMDA receptors in mechanisms of neurodegeneration/neuroprotection (reviewed by Hardingham and Bading, 2010). Alternatively, glutamate receptor types other than NMDA receptors may contribute to excitotoxic retinal degeneration.

Group-I metabotropic glutamate receptors (mGlu1 and mGlu5 receptors) are linked to NMDA receptors through a chain of anchoring proteins, and their activation enhances NMDA receptor function (Tu et al., 1999; Awad et al., 2000). mGlu1 and mGlu5 receptors are coupled to $G_{q/11}$. Their activation stimulates polyphosphoinositide (PI) hydrolysis, a transduction mechanism that leads to increased intracellular free Ca^{2+} and activation of protein kinase C (Nicoletti et al., 2011). Both effects might contribute to neuronal degeneration under pathological conditions. The role of mGlu1 and mGlu5 receptors in mechanisms of neurodegeneration/neuroprotection is discussed in numerous review articles (Nicoletti et al., 1999; Bruno et al., 2001, 2017).

In the retina, mGlu1 and mGlu5 receptors are present in ON-bipolar and amacrine cells. In addition, mGlu1 receptors are also found in RGCs (Koulen et al., 1997; Brandstätter et al., 1998; Yang, 2004). We found recently that mGlu1, but not mGlu5, receptors are coupled to PI hydrolysis in the mouse and bovine retina (Romano

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Abbreviations: mGlu, metabotropic glutamate receptor; MSG, monosodium glutamate; NAM, negative allosteric modulator; NMDA, N-methyl-D-aspartate; PI, polyphosphoinositide; PND, post-natal day; RGC, retinal ganglion cell; s.c., subcutaneous.

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et al., 2016). This suggests that, in pathological conditions, mGlu1 receptors might be involved in mechanisms of retinal degeneration. We tested this hypothesis using the monosodium glutamate (MSG) model of retinal degeneration, which is a milestone in the field of excitotoxicity offering one of the first demonstrations that excessive glutamate can be toxic to neurons. The model is based on systemic administration of MSG to pups in the first 9–10 days of postnatal life, when the blood–brain barrier and the blood–retina barriers are not yet fully developed. At postnatal day (PND)9/10 systemic MSG causes a severe retinal damage involving not only ganglion neurons, but also cells of the inner nuclear layer. MSG is less efficient in causing retinal degeneration at earlier postnatal days, and does not cause significant retinal damage at PND > 11 even at sublethal doses (Blood et al., 1969; Olney, 1969; Lowe et al., 1970). Using both genetic and pharmacological approaches, we now demonstrate that activation of mGlu1 receptors largely contributes to MSG-induced RGC death. As a pharmacological approach we treated mice systemically with compound JNJ16259685, a highly selective negative allosteric modulator (NAM) of mGlu1 receptors which blocks receptor function regardless of the concentrations of ambient glutamate (Lavreysen et al., 2004).

EXPERIMENTAL PROCEDURES

Animals

C57BL/6J mice (Charles River, Calco, Italy), *crv4* mice and their Balb/c wild-type littermates (provided by A.P.) were used in this study. *crv4* mice have a spontaneous recessive mutation consisting of a retrotransposon long terminal repeat (LTR) insertion that disrupts the splicing of the *Grm1* gene determining the absence of the mGlu1 receptor protein (Conti et al., 2006). *crv4* mice were generated by heterozygous breeding. Mouse genotyping was performed in DNA extracts from the mouse tail as reported previously (Conti et al., 2006). Pregnant dams had free access to food and water and were maintained in cages in an environmentally controlled room with a temperature of 22 °C, a humidity of 55%, and a 12-h light/dark cycle. The offspring was used at PND9, with no sex distinction.

We made all efforts to reduce the number of animals. Experiments were performed in conformity with the Statement for the Use of Animals in Ophthalmic and Vision Research and with the Italian law on Animal Care No. 26/2014 and the Directive 2010/63/EU. The study was approved by the local Animal Care and Use Committee of Neuromed Institute.

Experimental design

For toxicity experiments, C57BL/6J mice at PND9 (n = 3–9 mice per group) were treated as follows. Two groups received a subcutaneous (s.c.) injection of corn oil (Sigma–Aldrich, Milan, Italy), followed, 30 min later, by s.c. injection of either saline or MSG (3 g/kg; Sigma–Aldrich); two additional groups were treated with 3,4-dihydro-2 H-pyrano[2,3-b]quinolin-7-yl-(cis-4-methoxycyclohexyl)-methanone (JNJ16259685; Tocris Bioscience, Bristol, UK), dissolved in corn oil (2.5 mg/kg) followed, 30 min later, by a s.c. injection of either saline or MSG. In another set of experiments, four groups of *crv4* mice or their wild-type littermates (n = 5–7 mice per group) were injected s.c. with either saline or MSG (3 g/kg). Homozygous *crv4* and wild-type mice were genotyped prior to the experiment.

For behavioral analysis, two groups of 4 C57BL/6J mice at PND9 were treated s.c. with a single injection of either JNJ16259685 (2.5 mg/kg) or its vehicle. Behavioral analysis was performed 30 min and 18 h after injections. A total number of 59 mice were used in the study.

Immunohistochemical analysis and RGC counting

All mice were killed 18 h after drug injection. The eyes and cerebella were quickly removed. Cerebella were stored at −80 °C and then used for immunoblot analysis of mGlu1α receptors (see below). Eyes were placed in 4% (w/v) paraformaldehyde for 5 h at 4 °C. After extensive washing in water for 5 min, eyes were placed in 70% ethanol at 4 °C until paraffin inclusion.

Retinal sections (20 μm) were incubated overnight with polyclonal goat anti-Brn-3a antibody (1:100; sc-6026, Santa Cruz Biotechnology Inc., Dallas, TX, USA), for 1 h with secondary biotin-coupled anti-goat antibody (1:200; BA 9005, Vector Laboratories, Burlingame, CA, USA) and then for 1 h with Horseradish Peroxidase Streptavidin (1:100; SA-5004, Vector Laboratories). 3,3-diaminobenzidine tetrachloride (Sigma–Aldrich) was used for detection. Before incubation with primary antibody, sections were treated with citrate buffer, and heated in a microwave for 15 min for antigen retrieval. Negative control was performed without primary antibody.

Neuronal density values (number of neurons/mm^2) were calculated in retinal tissue slides stained with Brn-3a (sc-6026, Santa Cruz Biotechnology Inc.). Cells were counted at 100× magnification within a square area (disector), of 2500 μm^2 (50 × 50 μm). Cell counting was performed by an operator (D.B.) who was unaware of the treatments or mouse genotypes. The disectors were positioned within an area of interest (AOI) over the ganglion cell layer of the retinal slides. This AOI, hand-drawn by the operator, consistently started about 800 μm laterally to the emergence of the optic nerve. Within this area, the software (Image Pro Plus 6.2, Media Cybernetics, Inc.) randomly puts a series of disectors where ganglion cells were counted at high magnification (100×). The results were expressed as number of cell density per mm^2.

Measurements of cell number were not extended to other neuronal types of the retina (e.g., neurons of the inner nuclear layer) that could have been damaged by MSG administration (Olney, 1969).

Confocal microscopy analysis of mGlu1α receptors in the retina

For immunofluorescence analysis, 30-μm retinal sections were incubated with a mouse anti-mGlu1 primary

antibody (1:200, BD Bioscience, Milan, Italy). Serial sections were then incubated with a secondary antibody conjugated to Alexa Fluor 488 (1:200, Invitrogen, Carlsbad, CA, USA). Slides were coverslipped with mounting medium (Vector). Tissue sections were scanned using a LSM 5 Pascal confocal laser scanning microscope with a Zeiss ECPLAN-NEOFLUAR 40×/1.30 M27 oil immersion objective (Carl Zeiss Microimaging Inc.). We used a 488-nm argon laser to excite Alexa Fluor 488.

Western blot analysis of mGlu1α receptors in *crv4* and wild-type mice

Immunoblot analysis of mGlu1α receptors was carried in the cerebellum of *crv4* mice and their wild-type littermates as reported previously (Romano et al., 2016) using a mouse monoclonal anti-mGlu1α antibody (1:700, BD Biosciences).

Behavioral analysis

Analysis of motor behavior in PND9 pups treated with JNJ16259685 (2.5 mg/kg, s.c.) or its vehicle was performed as described by Feather-Schussler and Ferguson (2016).

All behavioral tests were performed 30 min and 18 h after injections, i.e., at 4.00 p.m. and 9.00 a.m. of the following day, respectively. The battery of motor tests included: (i) a 3-min ambulation test, in which the spontaneous motor activity of mice was scored as follows: 0 = absence of movements; 1 = crawling associated with asymmetric limb movements; 2 = symmetric but slow limb movements; and, 3 = fast movements; (ii) a righting reflex test, in which pups were placed on their backs and the time needed to turn over was recorded; (iii) a hindlimb suspension test, in which pups were placed vertically by their hind limbs inside a 50-ml plastic tube and hindlimb posture was scored as follows: 4 = hindlimb separation with a raised tail; 3 = hindlimbs are close to each other but they are still separated; 2 = hindlimbs are close and frequently touch each other; 1 = constant clasping of the hindlimbs with a raised tail; and 0 = persistent hindlimb clasping with a lowered tail; and (iv) a grip strength test used for the evaluation of grasp-reflex strength, in which mice were placed on a metal grid that was tilted slowly from horizontal to vertical position recording the angle of the grid when pups fell down.

Statistical analysis

Data are presented as medians and all individual data points in Fig. 1, and as means ± S.E.M. in Fig. 2 and Fig. 4. Statistical analysis of data shown in Fig. 1 was performed using the non-parametric Kruskal–Wallis test. Dunn's Multiple Comparison test was used to isolate the differences. Statistical analysis of data presented in Fig. 2 was performed by Student's *t*-test (*t* values are reported in the Results section and in the Figure legend). Statistical analysis of data shown in Fig. 4 was performed by a One-way ANOVA (F values are reported in the Results section and in the Figure legend); the Neumann–Keuls test was used as a *post hoc* test to isolate the differences. A p value < 0.05 was considered as statistically significant.

RESULTS

Pharmacological blockade of mGlu1 receptors is protective against MSG-induced neuronal death in the mouse retina

We performed immunohistochemical analysis using antibodies directed toward Brn-3a, a reliable marker for the identification and quantification of RGCs (Nadal-Nicolás et al., 2009). Accordingly, Brn-3a immunostaining was confined to RGCs and was not observed in other retinal layers (Figs. 1 and 4).

A single systemic injection of MSG (3 g/kg) to PND9 C57BL/6J mice caused extensive retinal degeneration reducing the number of Brn-3a$^+$ RGCs by >70% with respect to control mice treated with saline (Kruskal–Wallis' non parametric test and Dunn's Multiple Comparison test; $H_3 = 20.063$; $p < 0.001$) (Fig. 1A, B). Pre-treatment with the selective mGlu1 receptor NAM, JNJ16259685 (2.5 mg/ kg), reduced the damaging effect of MSG on retinal ganglion cells. In mice treated with MSG combined with JNJ16259685 the number of RGC was reduced by only <20%, and the resulting value was not significantly different from values obtained in all other groups of mice, including control mice. JNJ16259685 alone had no effect on RGC number (Kruskal–Wallis' non parametric test and Dunn's Multiple Comparison test) (Fig. 1A, B). These data suggest that activation of mGlu1 receptors largely contribute to excitotoxic RGC damage.

Evaluation of motor behavior in mice treated with JNJ16259685

To examine whether pharmacological blockade of mGlu1 receptors could affect motor behavior in PND9 pups, we used a battery of four behavioral tests that evaluate spontaneous motor activity, hindlimb posture, muscular strength, and the righting reflex (Feather-Schussler and Ferguson, 2016). At short time intervals after injection (30 min), systemic treatment with JNJ16259685 (2.5 mg/ kg, s.c.) did not cause significant changes in the ambulation score, hindlimb suspension score, and the grip strength test, and righting reflex (Student's *t*-test; $t_6 = 1.492$) (Fig. 2A). At 18 h after the treatment, no major changes in motor behavior were seen in mice receiving JNJ1629685, with the exception of a small but significant increase in the mouse performance in the grip strength test (Student's *t*-test; $t_6 = 2.771$; $p = 0.032$) (Fig. 2B).

MSG-induced retinal damage is attenuated in mice lacking mGlu1 receptors

To confirm a role for mGlu1 receptors in mechanisms of excitotoxic retinal degeneration, we used *crv4* mice and their wild-type littermates. The absence of mGlu1 receptor protein in *crv4* mice was further demonstrated by Western blot analysis of mGlu1α receptor protein in

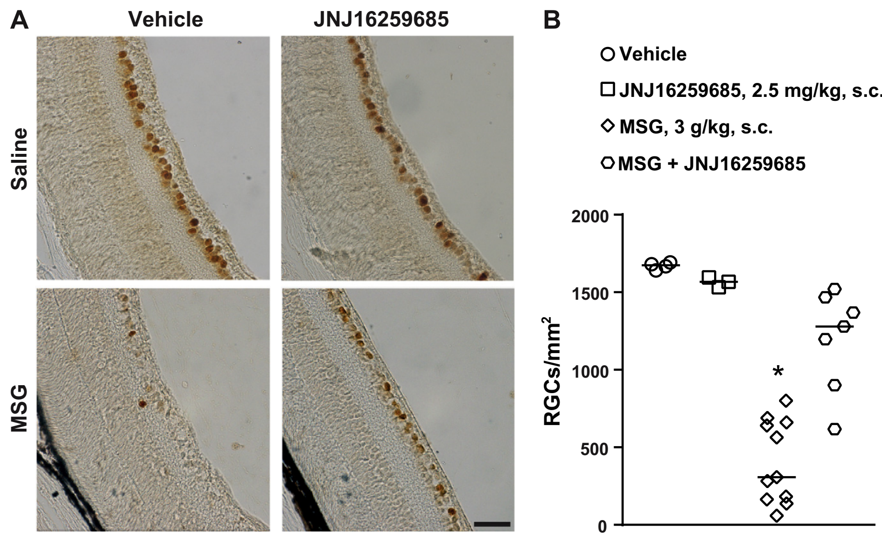

Fig. 1. Effect of systemic pharmacological blockade of mGlu1 receptors on MSG-induced RGC damage. Representative images of Brn-3a^{+} RGCs of mice treated s.c. with vehicle + saline, JNJ16259685 (2.5 mg/kg) + saline, vehicle + MSG (3 g/kg), and JNJ16259685 + MSG are shown in (A). Cell counts are shown in (B) as medians and all individual data points of 3–9 retinas per group (one retina per individual mouse). The equal variance parameter of a one-way ANOVA failed in the analysis perhaps owing to the limited size of the group treated with JNJ16259685 alone (n = 3). Thus, we used the non-parametric Kruskal–Wallis test followed by Dunn's Multiple Comparison test for statistical analysis (H_3 = 20.063; p < 0.001). Values obtained in mice treated with MSG alone (*) were significantly different with respect to values obtained in control mice (p < 0.001) or in mice treated with JNJ16259685 alone (p < 0.05). Values obtained in mice treated with MSG combined with JNJ16259685 did not significantly differ from data obtained in all other groups of mice. Treatment with JNJ16259685 alone had no significant effect on RGC counts with respect to control mice.

the cerebellum, a region in which mGlu1 receptors are expressed at high levels (Nicoletti et al., 2011). Immunoblots of mGlu1α receptor protein showed the expected band at 140 kDa corresponding to receptor monomers, and a higher molecular size band, corresponding to receptor dimers (Fig. 3A). The mGlu1α receptor was absent in the cerebellum of *crv4* mice, as expected (Fig.3A). We also examined mGlu1α receptor immunoreactivity in the retina of wild-type and *crv4* mice by confocal analysis. In wild-type mice, mGlu1α immunoreactivity was detected in the RGCs and inner plexiform layers (Fig.3B) in agreement with previous reports (Koulen et al., 1997; Dhingra and Vardi, 2012). mGlu1α immunoreactivity was not detected in the retina of *crv4* mice (Fig. 3B).

There was no difference in RGC number between *crv4* mice and their wild-type littermates (Fig. 4A, B). MSG administration to wild-type Balb/c littermates reduced RGC number to a lower extent than in C57BL/6J mice (compare Fig. 4 with Fig. 1), but the reduction was still highly significant (One-way ANOVA + Neumann–Keuls' *post-hoc* test; $F_{3,22}$ = 5.031; p < 0.05), indicating that excitotoxic retinal damage is strain-dependent. MSG-induced RGC degeneration was abolished in *crv4* mice (p < 0.05; Fig. 4A, B), confirming that, at least in the MSG model, activation of mGlu1 receptors is necessary for the induction/development of excitotoxic retinal damage.

DISCUSSION

Our data suggest that mGlu1 receptors are recruited in mechanisms of excitotoxic retinal degeneration and their activation is necessary for MSG-induced RCG death. This could not be automatically inferred from our knowledge of mGlu1 receptor signaling because the existing data on mGlu1 receptors and neurodegeneration are controversial. Most of the studies carried out in *in vitro* or *in vivo* models have shown a neuroprotective activity of mGlu1 receptor antagonists against excitotoxic or hypoxic/ischemic neuronal death (Bruno et al., 1999; Pellegrini-Giampietro et al., 1999; Battaglia et al., 2001; De Vry et al., 2001; Cozzi et al., 2002; Meli et al., 2002; Moroni et al., 2002; Murotomi et al., 2008, 2010; Smialowska et al., 2012; Li et al., 2013). However, mGlu1 receptor knockout mice were as vulnerable as their wild-type counterparts to focal cerebral ischemia and kainate-induced neurotoxicity (Ferraguti et al., 1997), and pharmacological activation of mGlu1 receptors was found to protect motor neurons against kainate-induced toxicity (Valerio et al., 2002). Thus, mGlu1 receptors can be either neurotoxic or neuroprotective depending upon the cellular context and the experimental paradigm of neurotoxicity. For example, Michel Baudry and his colleagues have shown that mGlu1 receptors may activate either a neuroprotective signal (the phosphatidylinositol-3-kinase pathway) or a toxic signal (intracellular Ca^{2+} release), and the concomitant activation of NMDA receptors shifts the balance toward the neurotoxic signal (Xu et al., 2007; Zhou et al., 2009). In cultured neurons, mGlu1 receptors behave as a «dependence receptors», supporting cell survival in response to agonist activation, and causing apoptotic death in the absence of glutamate (Pshenichkin et al., 2008). Another variable is the heterodimerization of mGlu1 receptors with mGlu5 receptors (Doumazane et al., 2011), which are also coupled to PI hydrolysis and have a dual role in mechanisms of neu rodegeneration/neuroprotection (Nicoletti et al., 1999).

At least the latter component is absent in the retina, where only mGlu1 receptors appear to be coupled to PI hydrolysis (with the detection limits of the PI assay). Accordingly, using both bovine retinal slices and intact mouse retinas, we were able to demonstrate that stimulation of PI hydrolysis by the mGlu1/5 receptor agonist, 3,5-dihydroxyphenylglycine (DHPG), was sensitive to mGlu1 receptor blockade, but was not

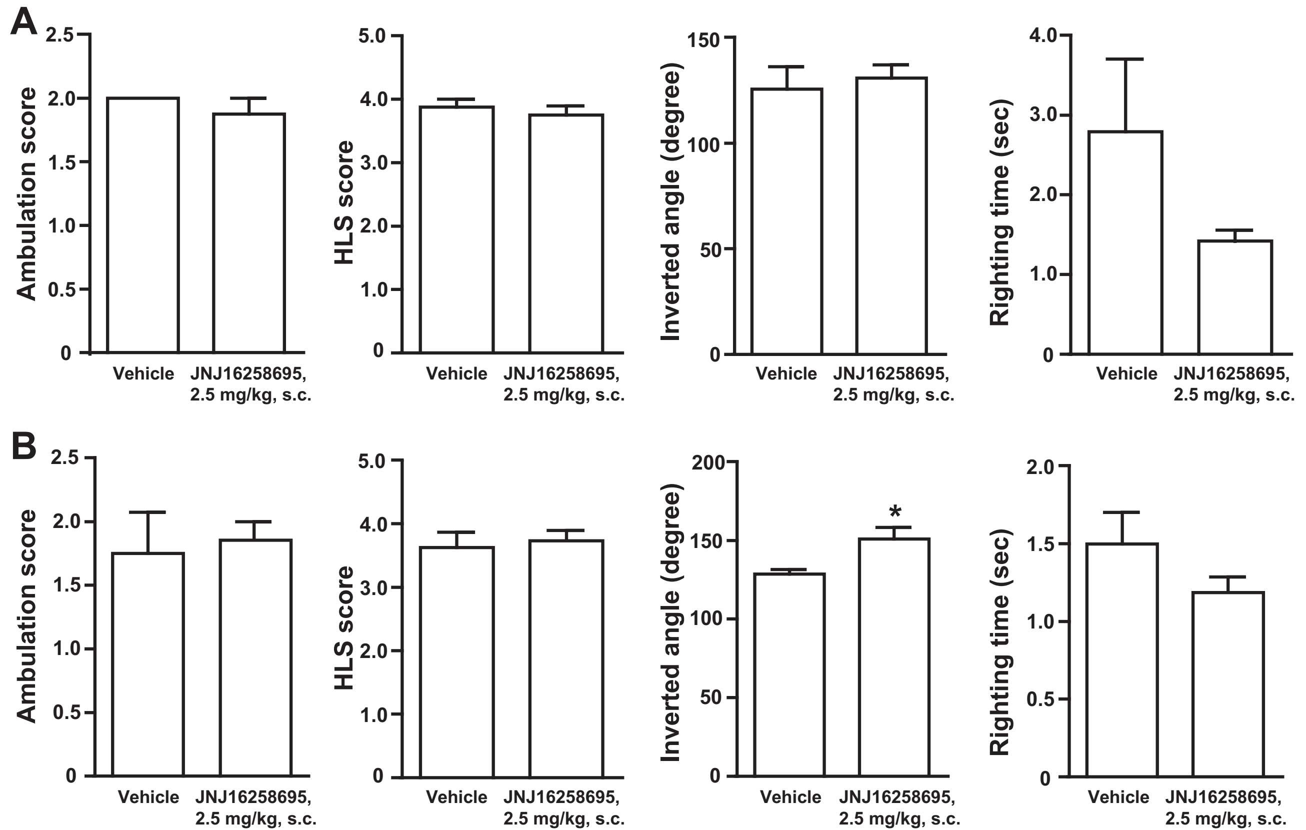

Fig. 2. Effect of JNJ16259685 treatment on motor behavior in PND9 pups. The effect of systemic JNJ16259685 treatment (2.5 mg/kg, s.c.) on spontaneous motor activity (ambulation score), hindlimb posture (hindlimb suspension (HLS) score), grip strength test (inverted angle), and righting reflex (righting time) at 30 min and 18 h after injection is shown in (A) and (B), respectively. Values are means ± S.E.M. of 4 mice per group. $^{*}p < 0.05$ (Student's t test; $t_6 = 2.771$) vs. the corresponding value of mice treated with vehicle.

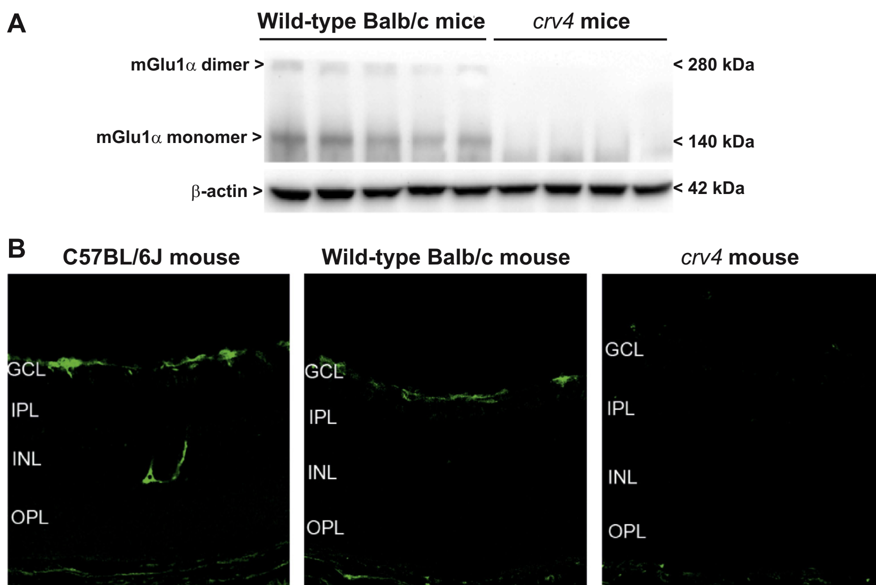

Fig. 3. Lack of mGlu1 receptors in the cerebellum and retina of *crv4* mice used for toxicity experiments. Western blot analysis of mGlu1α receptor protein in the cerebellum of wild-type littermates of Balb/c strain and *crv4* mice is shown in (A). The representative immunoblot (n = 4–5 per group) shows the absence of mGlu1α receptor in *crv4* mice, as expected. Representative confocal images of mGlu1α receptor protein in the retina of *crv4* mice and their wild-type littermates of Balb/c strain are shown in (B). mGlu1α receptor expression in C57BL/6J mice is also shown for comparison. Note that in wild-type mice mGlu1α receptor immunoreactivity was mainly detected in the ganglion cell layer (GCL), and that no immunoreactivity was detected in *crv4* mice.

influenced by mGlu5 receptor blockade. In addition, we found no PI response to DHPG in retinas of *crv4* mice lacking mGlu1 receptors, whereas the response was intact in retinas of mGlu5 receptor knockout mice (Romano et al., 2016). Thus, the retina offers a unique example of a tissue in which the PI response to DHPG is exclusively mediated by mGlu1 receptors. Our present findings indicate that mGlu1 receptors are key players in mechanisms of RGC degeneration triggered by toxic concentrations of glutamate. In the interpretation of data obtained after systemic treatment with JNJ16259685 we cannot exclude that the drug was protective by blocking mGlu1 receptors outside the retina. However, this is unlikely because, at least to our knowledge, brain structures that express significant levels of mGlu1 receptors (e.g., the cerebellum, thalamus, olfactory bulb, and hippocampal dentate gyrus) do not send projections to the retina. We could not

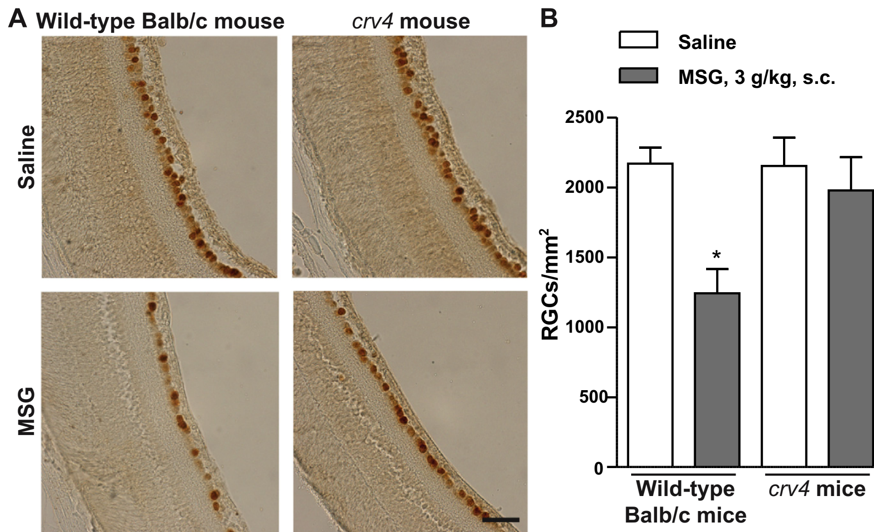

Fig. 4. *crv4* mice lacking mGlu1α receptors are protected against MSG-induced RGC damage. Representative images of Brn-3a$^+$ RGCs of wild-type and *crv4* mice treated s.c. with vehicle or JNJ16259685 (2.5 mg/kg) are shown in (A). Cell counts are shown in (B), where values are means + S.E.M. of 5–7 retinas per group (one retina per individual mouse). $^*p < 0.05$ (One-way ANOVA + Neumann–Keuls' *post hoc* test) vs. all other groups. One-way ANOVA: $F_{3,22} = 5.03$; $p = 0.0083$.

perform direct injections of JNJ16259685 into the vitreous of pups for a series of technical reasons. For example, eyelids are still closed at PND9 in mice, and intravitreal injections require a surgical cut of the eyelid. In addition, the whole procedure requires deep anesthesia, which is expected to have a significant impact on the study of the role played by a glutamate receptor (the mGlu1 receptor) in excitotoxic retinal degeneration. Our findings have potential translational value in the treatment of prematurity-induced retinal damage, in which excitotoxicity largely contributes to the pathophysiology of RGC death (see Introduction and References therein). Pituitary adenylyl cyclase activating peptide (PACAP) and the potassium channel activator, diazoxide, have shown neuroprotective activity in the MSG model in rats (Tamás et al., 2004; Rácz et al., 2007; Atlasz et al., 2007, 2008). However, both compounds were tested after intravitreal administration, and whether systemic administration of PACAP or diazoxide may confer neuroprotection against retinal damage is unknown. JNJ16259685 shows central bioavailability in rodents (Lavreysen et al., 2004), and, because of its hydrophobicity, is expected to cross the blood–retina barrier and reach sufficient concentrations to achieve neuroprotection in the developing human retina. By definition, NAMs bind to an allosteric region of mGlu1 receptors, and, therefore, they inhibit mGlu1 receptor activation regardless of the ambient concentrations of glutamate. Thus, JNJ16259685 and other mGlu1 receptor NAMs might be potential candidates for the treatment of prematurity-induced retinal degeneration. From a therapeutic perspective, it was important to examine the effect of systemic treatment with JNJ16259685 on motor behavior in PND9 mice. Interestingly, the drug did not cause detectable abnormalities in motor function after 30 min and 18 h, and even improved the performance in the grip strength test at 18 h. This was unexpected because mGlu1 receptor blockade is known to impair motor learning and motor coordination, two functions that are mediated by mGlu1 receptors expressed by cerebellar Purkinje cells (Aiba et al., 1994; Conquet et al., 1994; Ichise et al., 2000; Ohtani et al., 2014; Kano and Watanabe, 2017). However, mGlu1 receptors are still expressed at low levels in the cerebellum of PND9 mice, and begin to control cerebellar maturation and function after PND12 (reviewed by Kano and Watanabe, 2017). The developmental pattern of expression of mGlu1 receptors in the human cerebellum is unknown, and, therefore, we cannot exclude that the use of mGlu1 receptor NAMs for the treatment of prematurity-induced retinal degeneration may have an impact on motor learning and motor performance in humans.

Our data also encourage the study of mGlu1 receptor NAMs as potential disease modifiers in the treatment of glaucoma and other chronic neurodegenerative disorders of the retina. The study, however, requires a chronic systemic treatment with mGlu1 receptor NAMs, which, in this case, might have negative consequences on motor learning and motor coordination by restraining the activity of mGlu1 receptors in cerebellar Purkinje cells (Aiba et al., 1994; Conquet et al., 1994; Ichise et al., 2000; Ohtani et al., 2014; Kano and Watanabe, 2017). Thus, accurate pharmacological studies in experimental animal models of glaucoma and other degenerative eye disorders are needed to establish whether mGlu1 receptor blockade might be considered as a valuable and safe strategy to slow the progression of these disorders.

CONFLICT OF INTEREST

The authors do not have any conflict of interest.

REFERENCES

Aiba A, Kano M, Chen C, Stanton ME, Fox GD, Herrup K, Zwingman TA, Tonegawa S (1994) Deficient cerebellar long-term depression and impaired motor learning in mGluR1 mutant mice. Cell 79:377–388.

Atlasz T, Babai N, Kiss P, Reglodi D, Tamás A, Szabadfi K, Tóth G, Hegyi O, Lubics A, Gábriel R (2007) Pituitary adenylate cyclase activating polypeptide is protective in bilateral carotid occlusion-induced retinal lesion in rats. Gen Comp Endocrinol 153:108–114.

Atlasz T, Szabadfi K, Kiss P, Babai N, Koszegi Z, Tamas A, Reglodi D, Gabriel R (2008) PACAP-mediated neuroprotection of neurochemically identified cell types in MSG- induced retinal degeneration. J Mol Neurosci 36:97–104.

Awad H, Hubert GW, Smith Y, Levey AI, Conn PJ (2000) Activation of metabotropic glutamate receptor 5 has direct excitatory effects

and potentiates NMDA receptor currents in neurons of the subthalamic nucleus. J Neurosci 20:7871–7879.
Battaglia G, Bruno V, Pisani A, Centonze D, Catania MV, Calabresi P, Nicoletti F (2001) Selective blockade of type-1 metabotropic glutamate receptors induces neuroprotection by enhancing gabaergic transmission. Mol Cell Neurosci 17:1071–1083.
Blood FR, Oser BL, White PL, Olney JW (1969) Monosodium glutamate. Science 165:1028–1029.
Brandstätter JH, Koulen P, Wässle H (1998) Diversity of glutamate receptors in the mammalian retina. Vision Res 38:1385–1397.
Bruno V, Battaglia G, Kingston A, O'Neill MJ, Catania MV, Di Grezia R, Nicoletti F (1999) Neuroprotective activity of the potent and selective mGlu1a metabotropic glutamate receptor antagonist, (+)-2-methyl-4 carboxyphenylglycine (LY367385): comparison with LY357366, a broader spectrum antagonist with equal affinity for mGlu1a and mGlu5 receptors. Neuropharmacology 38:199–207.
Bruno V, Battaglia G, Copani A, Cespédes VM, Galindo MF, Ceña V, Sánchez-Prieto J, Gasparini F, Kuhn R, Flor PJ, Nicoletti F (2001) An activity-dependent switch from facilitation to inhibition in the control of excitotoxicity by group I metabotropic glutamate receptors. Eur J Neurosci 13:1469–1478.
Bruno V, Caraci F, Copani A, Matrisciano F, Nicoletti F, Battaglia G (2017) The impact of metabotropic glutamate receptors into active neurodegenerative processes: a "dark side" in the development of new symptomatic treatments for neurologic and psychiatric disorders. Neuropharmacology 115:180–192.
Casson RJ (2006) Possible role of excitotoxicity in the pathogenesis of glaucoma. Clin Exp Ophthalmol 34:54–63.
Chidlow G, Wood JP, Casson RJ (2007) Pharmacological neuroprotection for glaucoma. Drugs 67:725–759.
Conquet F, Bashir ZI, Davies HC, Daniel H, Ferraguti F, Bordi F, Franz-Bacon K, Reggiani A, Matarese V, Condé F, Collingridge GL, Crépe LF (1994) Motor deficit and impairment of synaptic plasticity in mice lacking mGluR1. Nature 372:237–243.
Conti V, Aghaie A, Cilli M, Martin N, Caridi G, Musante L, Candiano G, Castagna M, Fairen A, Ravazzolo R, Guenet JL, Puliti A (2006) Crv4, a mouse model for human ataxia associated with kypho-scoliosis caused by an mRNA splicing mutation of the metabotropic glutamate receptor 1 (Grm1). Int J Mol Med 18:593–600.
Cozzi A, Meli E, Carlà V, Pellicciari R, Moroni F, Pellegrini-Giampietro DE (2002) Metabotropic glutamate 1 (mGlu1) receptor antagonists enhance GABAergic neurotransmission: a mechanism for the attenuation of post-ischemic injury and epileptiform activity? Neuropharmacology 43:119–130.
De Vry J, Horváth E, Schreiber R (2001) Neuroprotective and behavioral effects of the selective metabotropic glutamate mGlu (1) receptor antagonist BAY 36–7620. Eur J Pharmacol 428:203–214.
Dhingra A, Vardi N (2012) "mGlu Receptors in the Retina" – WIREs Membrane Transport and Signaling. Wiley Interdiscip Rev Membr Transp Signal 1:641–653.
Doumazane E, Scholler P, Zwier JM, Trinquet E, Rondard P, Pin JP (2011) A new approach to analyze cell surface protein complexes reveals specific hetero-dimeric metabotropic glutamate receptors. FASEB J 25:66–77.
Feather-Schussler DN, Ferguson TS (2016) A battery of motor tests in a neonatal mouse model of cerebral palsy. J Vis Exp: JoVE 117:53569.
Ferraguti F, Pietra C, Valerio E, Corti C, Chiamulera C, Conquet F (1997) Evidence against a permissive role of the metabotropic glutamate receptor 1 in acute excitotoxicity. Neuroscience 79:1–5.
Ferreira IL, Duarte CB, Carvalho AP (1996) Ca2+ influx through glutamate receptor-associated channels in retina cells correlates with neuronal cell death. Eur J Pharmacol 302:153–162.
Hardingham GE, Bading H (2010) Synaptic versus extrasynaptic NMDA receptor signalling: implications for neurodegenerative disorders. Nat Rev Neurosci 11:682–696.
Hernández C, Simó R (2012) Neuroprotection in diabetic retinopathy. Curr Diabetes Rep 12:329–337.
Hinton DR, Sadun AA, Blanks JC, Miller CA (1986) Optic-nerve degeneration in Alzheimer's disease. N Engl J Med 315:485–487.
Ichise T, Kano M, Hashimoto K, Yanagihara D, Nakao K, Shigemoto R, Katsuki M, Aiba A (2000) MGluR1 in cerebellar Purkinje cells essential for long-term depression, synapse elimination, and motor coordination. Science 288:1832–1835.
Kano M, Watanabe T (2017) Type-1 metabotropic glutamate receptor signaling in cerebellar Purkinje cells in health and disease. F1000 Res 6:416.
Kaur C, Foulds WS, Ling EA (2008) Hypoxia-ischemia and retinal ganglion cell damage. Clin Ophthalmol 2:879–889.
Koulen P, Kuhn R, Wässle H, Brandstätter JH (1997) Group I metabotropic glutamate receptors mGluR1alpha and mGluR5a: localization in both synaptic layers of the rat retina. J Neurosci 17:2200–2211.
Lavreysen H, Wouters R, Bischoff F, Nóbrega Pereira S, Langlois X, Blokland S, Somers M, Dillen L, Lesage AS (2004) JNJ16259685, a highly potent, selective and systemically active mGlu1 receptor antagonist. Neuropharmacology 47:961–972.
Li H, Zhang N, Sun G, Ding S (2013) Inhibition of the group I mGluRs reduces acute brain damage and improves long-term histological outcomes after photothrombosis-induced ischaemia. ASN Neuro 5:195–207.
Lipton SA (2003) Possible role for memantine in protecting retinal ganglion cells from glaucomatous damage. Surv Ophthalmol 1:38–46.
Lowe CU, Zavon MR, Olney JW, Sharpe LG (1970) Monosodium glutamate: specific brain lesion questioned. Science 167:1016–1017.
Meli E, Picca R, Attucci S, Cozzi A, Peruginelli F, Moroni F, Pellegrini-Giampietro DE (2002) Activation of mGlu1 but not mGlu5 metabotropic glutamate receptors contributes to postischemic neuronal injury in vitro and in vivo. Pharmacol Biochem Behav 73:439–446.
Moroni F, Attucci S, Cozzi A, Meli E, Picca R, Scheideler MA, Pellicciari R, Noe C, Sarichelou I, Pellegrini-Giampietro DE (2002) The novel and systemically active metabotropic glutamate 1 (mGlu1) receptor antagonist 3-MATIDA reduces post-ischemic neuronal death. Neuropharmacology 42:741–751.
Murotomi K, Takagi N, Takayanagi G, Ono M, Takeo S, Tanonaka K (2008) MGluR1 antagonist decreases tyrosine phosphorylation of NMDA receptor and attenuates infarct size after transient focal cerebral ischemia. J Neurochem 105:1625–1634.
Murotomi K, Takagi N, Mizutani R, Honda TA, Ono M, Takeo S, Tanonaka K (2010) MGluR1 antagonist decreased NADPH oxidase activity and superoxide production after transient focal cerebral ischemia. J Neurochem 114:1711–1719.
Nadal-Nicolás FM, Jiménez-López M, Sobrado-Calvo P, Nieto-López L, Cánovas-Martínez I, Salinas-Navarro M, Vidal-Sanz M, Agudo M (2009) Brn3a as a marker of retinal ganglion cells: qualitative and quantitative time course studies in naive and optic nerve-injured retinas. Invest Ophthalmol Vis Sci 50:3860–3868.
Nicoletti F, Bruno V, Catania MV, Battaglia G, Copani A, Barbagallo G, Ceña V, Sanchez-Prieto J, Spano PF, Pizzi M (1999) Group-I metabotropic glutamate receptors: hypotheses to explain their dual role in neurotoxicity and neuroprotection. Neuropharmacology 38:1477–1484.
Nicoletti F, Bockaert J, Collingridge GL, Conn PJ, Ferraguti F, Schoepp DD, Wroblewski JT, Pin JP (2011) Metabotropic glutamate receptors: from the workbench to the bedside. Neuropharmacology 60:1017–1041.
Niwa M, Aoki H, Hirata A, Tomita H, Green PG, Hara A (2016) Retinal cell degeneration in animal models. Int J Mol Sci 17(1).
Ohtani Y, Miyata M, Hashimoto K, Tabata T, Kishimoto Y, Fukaya M, Kase D, Kassai H, Nakao K, Hirata T, Watanabe M, Kano M, Aiba A (2014) The synaptic targeting of mGluR1 by its carboxyl-terminal domain is crucial for cerebellar function. J Neurosci 34:2702–2712.
Olney JW (1969) Glutamate-induced retinal degeneration in neonatal mice. Electron microscopy of the acutely evolving lesion. J Neuropathol Exp Neurol 28:455–474.

Osborne NN (2009) Recent clinical findings with memantine should not mean that the idea of neuroprotection in glaucoma is abandoned. Acta Ophthalmol 87:450–454.

Pellegrini-Giampietro DE, Peruginelli F, Meli E, Cozzi A, Albani-Torregrossa S, Pellicciari R, Moroni F (1999) Protection with metabotropic glutamate 1 receptor antagonists in models of ischemic neuronal death: time-course and mechanisms. Neuropharmacology 38:1607–1619.

Pshenichkin S, Dolińska M, Klauzińska M, Luchenko V, Grajkowska E, Wroblewski JT (2008) Dual neurotoxic and neuroprotective role of metabotropic glutamate receptor 1 in conditions of trophic deprivation – possible role as a dependence receptor. Neuropharmacology 55:500–508.

Rácz B, Gallyas Jr F, Kiss P, Tamás A, Lubics A, Lengvári I, Röth E, Tóth G, Hegyi O, Verzál Z, Fabricsek C, Reglódi D (2007) Effects of pituitary adenylate cyclase activating polypeptide (PACAP) on the PKA-Bad-14-3-3 signaling pathway in glutamate-induced retinal injury in neonatal rats. Neurotox Res 12:95–104.

Romano MR, Di Menna L, Scarselli P, Mascio G, Madonna M, Notartomaso S, Puliti A, Bruno V, Battaglia G, Nicoletti F (2016) Type-1, but not type-5, metabotropic glutamate receptors are coupled to polyphosphoinositide hydrolysis in the retina. Neurochem Res 41:924–932.

Seki M, Lipton SA (2008) Targeting excitotoxic/free radical signaling pathways for therapeutic intervention in glaucoma. Prog Brain Res 173:495–510.

Śmiaowska M, Goembiowska K, Kajta M, Zięba B, Dziubina A, Domin H (2012) Selective mGluR1 antagonist EMQMCM inhibits the kainate-induced excitotoxicity in primary neuronal cultures and in the rat hippocampus. Neurotox Res 21:379–392.

Tamás A, Gábriel R, Rácz B, Dénes V, Kiss P, Lubics A, Lengvári I, Reglodi D (2004) Effects of pituitary adenylate cyclase activating polypeptide in retinal degeneration induced by monosodium-glutamate. Neurosci Lett 372:110–113.

Tenhula WN, Xu SZ, Madigan MC, Heller K, Freeman WR, Sadun AA (1992) Morphometric comparisons of optic nerve axon loss in acquired immunodeficiency syndrome. Am J Ophthalmol 113:14–20.

Tu JC, Xiao B, Naisbitt S, Yuan JP, Petralia RS, Brakeman P, Doan A, Aakalu VK, Lanahan AA, Sheng M, Worley PF (1999) Coupling of mGluR/Homer and PSD-95 complexes by the Shank family of postsynaptic density proteins. Neuron 23:583–592.

Valerio A, Ferrario M, Paterlini M, Liberini P, Moretto G, Cairns NJ, Pizzi M, Spano P (2002) Spinal cord mGlu1a receptors: possible target for amyotrophic lateral sclerosis therapy. Pharmacol Biochem Behav 73:447–454.

Xu W, Wong TP, Chery N, Gaertner T, Wang YT, Baudry M (2007) Calpain-mediated mGluR1alpha truncation: a key step in excitotoxicity. Neuron 53:399–412.

Yang XL (2004) Characterization of receptors for glutamate and GABA in retinal neurons. Prog Neurobiol 73:127–150.

Zhou M, Xu W, Liao G, Bi X, Baudry M (2009) Neuroprotection against neonatal hypoxia/ischemia-induced cerebral cell death by prevention of calpain-mediated mGluR1alpha truncation. Exp Neurol 218:75–82.

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